HIV Detection from Enzo Diagnostics

The Enzo Microplate Assay*for HIV DNA

for direct detection and quantitation of HIV virus

The ***Enzo Microplate Assay for HIV DNA*** is an easy-to-use, rapid and nonradioactive kit for detecting HIV DNA. It has wide application to research in epidemiology, diagnosis and treatment. The straightforward assay procedure is carried out in a microtiter plate or microwell strips. The generation of a color, which can be easily measured, indicates a positive reaction.

The ***Enzo Microplate Assay*** provides materials for the colorimetric detection of HIV proviral DNA or viral RNA which has been reverse transcribed. When used in combination with gene amplification techniques, this test provides several advantages over current procedures. Unlike Southern blots and other hybridization techniques, no radioactivity, no electrophoresis and no film are used. No specialized equipment, other than a standard microplate reader, which is present in most medical research labs, is required. In fact, for plus/minus determinations the results can be read by eye. The assay format uses either a microtiter plate, where up to 96 assays (including controls) can be run, or well strips, where 8 assays per strip (including controls) can be run. Assay of the amplified DNA requires less than three hours. Thus, when combined with the amplification step, samples can be processed in a single day.

The ***Microplate Assay*** detects HIV proviral DNA with excellent sensitivity. The data presented in Figure 2 show that fewer than 10 HIV proviral sequences can be detected. Furthermore, when the assay is performed using a standard curve of HIV DNA copies, quantitative virus measurements can be done. Thus, the ***Microplate Assay*** is readily applicable to studies measuring the effect of drug treatments on virus concentration, virus concentration during the course of infection, virus concentration in animal model studies and numerous other studies where virus quantitation is a critical parameter.

Direct and quantitative HIV detection offers a distinct advantage over serological assays. The identification of provirus DNA can be made independent of the presence or absence of antibodies, which appear several months after initial infection. Thus, this technique offers researchers the potential for identifying proviral DNA in instances where HIV positivity can not be identified adequately by any of the current means available as, for example, in samples from HIV positive individuals who have not seroconverted.

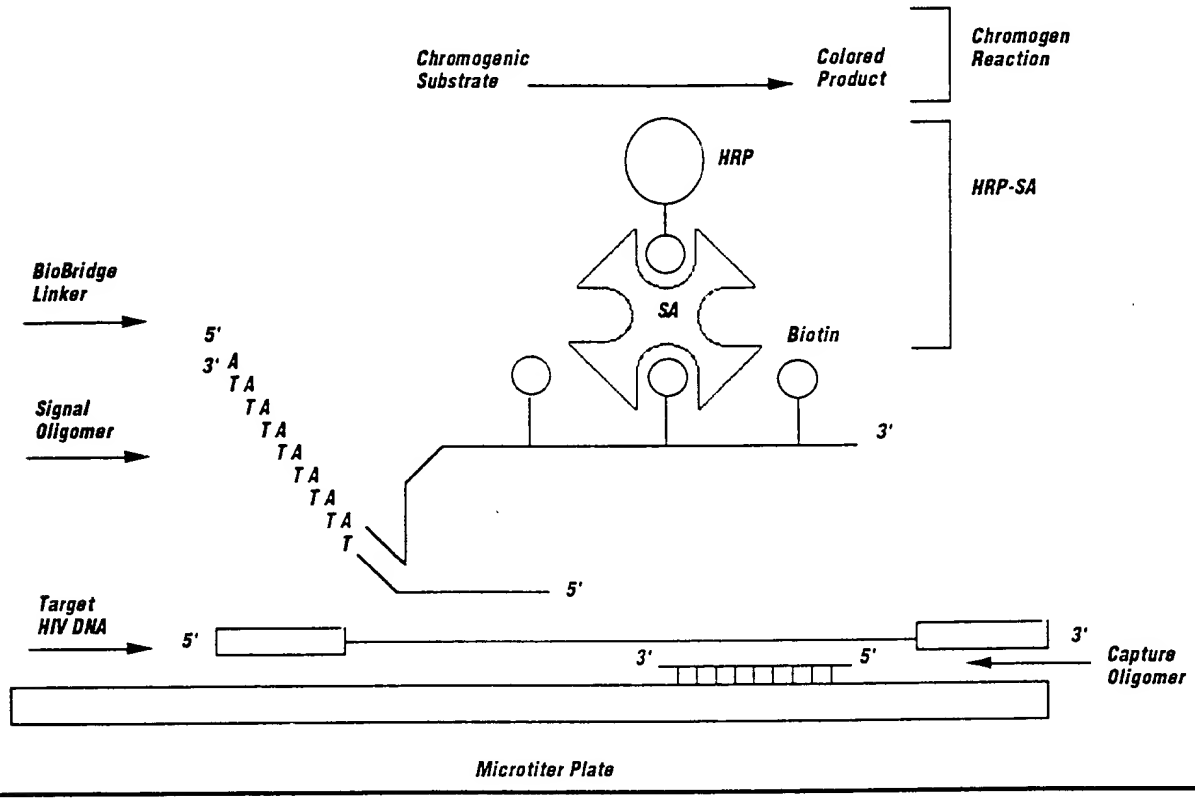
Reference:

Cook, A.F., Vuocolo, E. and Brakel, C.L. (1988) Synthesis and hybridization of a series of biotinylated oligonucleotides. Nuc. Acid Res. 16:4077-4095.

The Enzo Microplate Assay for HIV DNA
Catalog No. 46330

**U.S. Patent Nos. 4,711,955, 4994,373 and patents pending*
The Enzo Microplate Assay is for research use only

Figure 1.
Schematic Representation of the Enzo Microplate Assay for HIV DNA



PROCEDURE

The ***Enzo HIV Microplate Assay Kit*** provides materials for the colorimetric detection of nucleic acid containing the gag region. The method is based on a two probe hybridization procedure performed in a 96-well microtiter plate or a microwell strip. HIV DNA can be assayed directly if there is sufficient target DNA present, or it can be assayed indirectly in procedures employing target amplification. The nonradioactive procedure involves hybridization of target nucleic acid to a well-bound capture probe followed by binding of a biotinylated signaling probe to the captured target. Immobilized target DNA is then visualized by reaction with a biotin-binding signal generation complex of streptavidin (SA) and horseradish peroxidase (HRP). A positive reaction is indicated by generation of color which can be measured by a microplate reader commonly used in the laboratory. Using this format, 10^7 to 10^8 copies of target sequences can be detected.

DENATURE SAMPLE
Incubate sample with buffer for 15 minutes to denature the target nucleic acid sequences.

HYBRIDIZE TO WELL-BOUND CAPTURE PROBE
After rinsing the wells, add sample to each well and incubate for 90-120 minutes.

REACTION WITH SIGNAL PROBE
After the sample DNA is hybridized to the capture probe, signal probe is added and incubated for 15 minutes.

ADDITION OF LINKER
After rinsing the wells, add the linker (which supplies the biotin) to each well and incubate for 10 minutes.

DETECTION
Add the streptavidin-horseradish peroxidase complex, and then add the chromogen/substrate to generate color.

- *A positive result appears as a blue color which turns to yellow upon addition of the stop solution.*
- *Results may be quantified by reading OD at 450.*

HIV Detection

from Enzo Diagnostics

for direct detection and quantitation of the human immunodeficiency virus

The Enzo Microplate Assay* for HIV DNA

Catalog No. 46330

The ***Enzo Microplate Assay for HIV DNA*** is an easy-to-use, rapid and nonradioactive kit for detecting HIV-1 DNA. It has wide application to research in epidemiology, diagnosis and treatment. The straightforward assay procedure is carried out in a microtiter plate. The generation of a color, which can be easily measured, indicates a positive reaction.

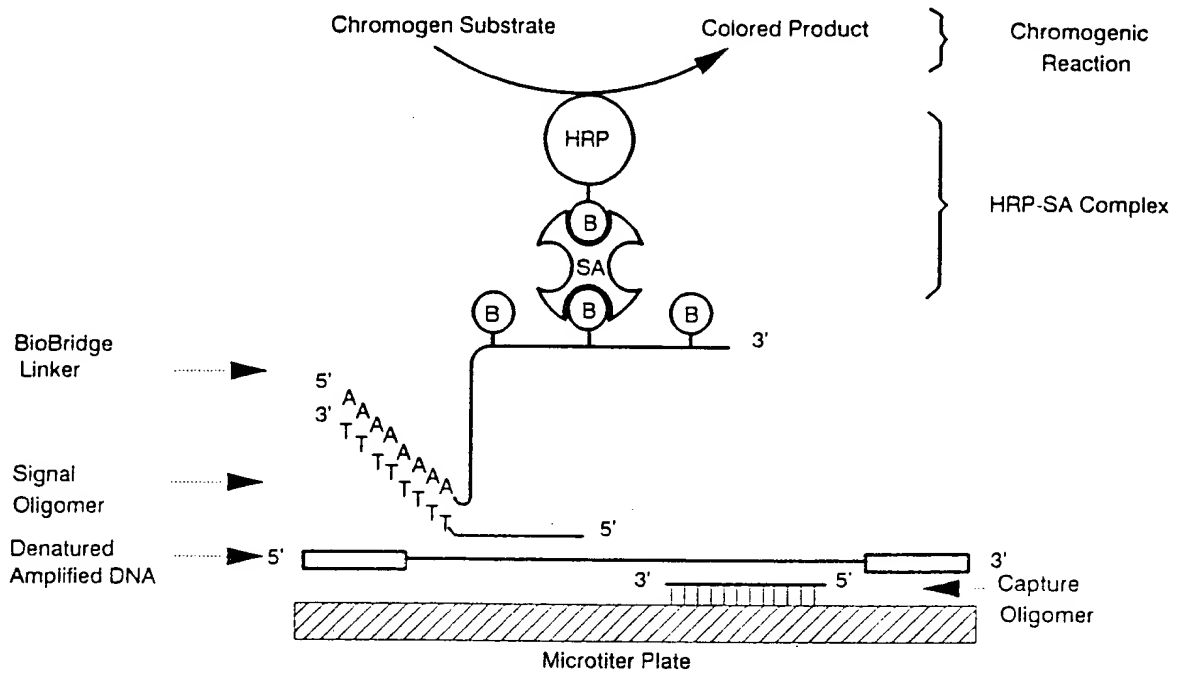
The ***Enzo Microplate Assay for HIV DNA*** provides materials for the colorimetric detection of HIV proviral DNA or viral RNA that has been reverse transcribed. When used in combination with gene amplification techniques, this test provides several advantages over current procedures. Unlike Southern blots and other hybridization techniques, no radioactivity, no electrophoresis and no film are used. No specialized equipment, other than a standard microplate reader, which is present in most medical research labs, is required. In fact, for plus/minus determinations the results can be read by eye. The assay format uses a microtiter plate in which up to 96 assays (including controls) can be run. Assay of the amplified DNA requires less than three hours. Thus, when combined with the amplification step, samples can be processed in a single day.

The **Microplate Assay** detects HIV DNA with excellent sensitivity. The data presented in Figure 2 show that fewer than 10 proviral sequences can be detected. Furthermore, when the assay is performed using a standard curve of HIV DNA copies, quantitative virus measurements can be done. Thus, the **Microplate Assay** is readily applicable to studies measuring the effect of drug treatments on virus concentration, virus concentration during the course of infection, virus concentration in animal model studies and numerous other studies where virus quantitation is a critical parameter.

Quantitative HIV detection offers a distinct advantage over serological assays. The identification of proviral DNA can be made independent of the presence or absence of antibodies, that appear several months after initial infection. This technique offers researchers the potential for identifying proviral DNA in instances where HIV positivity can not be identified adequately in samples from HIV positive individuals who have not seroconverted.

U.S. Patent Nos. 4,711,955; 4,994,373; and 5,328,824; EP 0 063879 B1; EP 0 117440 B1; and EP 0 122614 B1; Canadian Patent Nos. 1,219,824; 1,309,672; 1,254,525; and patents pending

Figure 1
Schematic Representation of the Enzo Microplate Assay for HIV DNA



Summary and Use

The ***Enzo Microplate Assay Kit*** provides materials for the colorimetric detection of nucleic acid containing the *gag* region. As depicted in Figure 1, the method is based on a two probe hybridization procedure performed in a 96-well microtiter plate. HIV DNA can be assayed directly if there is sufficient target DNA present, or it can be assayed indirectly in procedures employing target amplification. The nonradioactive procedure involves hybridization of target nucleic acid to a well-bound capture probe followed by binding of a biotinylated signaling probe to the captured target. Immobilized target DNA is then visualized by reaction with a biotin-binding signal generation complex of streptavidin (SA) and horseradish peroxidase (HRP). A positive reaction is indicated by generation of color that can be measured by a microplate reader commonly used in the laboratory. Using this format 10^7 to 10^8 copies of target sequences can be detected. With amplified samples, the ultimate sensitivity is dependent upon the amplification efficiency. As shown in Figure 2, after 35 rounds of amplification, fewer than 10 copies of HIV DNA could be detected.

The assay utilizes several unique features. The use of probe pairs increases the specificity of the assay. Two independent hybridization events are required to generate a signal. This type of assay has been found to be insensitive to the presence of cellular components other than the target DNA sequence.

The Enzo Microplate Assay is for research use only

Since the assay is nonradioactive, the components are safe and stable, and the assay can be carried out manually or using devices developed for the automated processing of microtiter plate-based assays.

Procedure

Denature Sample
Incubate sample with buffer for 15 minutes to denature the target nucleic acid sequences.

Hybridize to Well-Bound Capture Probe
After rinsing the wells, add sample to each well and incubate for 90-120 minutes.

Reaction with Signal Probe
After the sample DNA is hybridized to the capture probe, signal probe is added and incubated for 15 minutes.

Addition of Linker
After rinsing the wells, add the linker (which supplies the biotin) to each well and incubate for 10 minutes.

Detection
Add the streptavidin-horseradish peroxidase complex and then add the chromogen/substrate to generate color.

- *A positive result appears as a blue color which turns to yellow upon addition of the stop solution.*
- *Results may be quantified by reading OD at 450 nm.*

Summary of Collaborative Studies

To investigate the performance of the microplate assay in clinical specimens, studies were undertaken with the Division of HIV/AIDS at the Centers for Disease Control in Atlanta. The following is a summary of some of these studies.

The assay for HIV DNA sequences (Figure 1) was adapted from the colorimetric microplate hybridization assay described by Cook *et al.*(1) Oligonucleotide sequences were chosen from two relatively conserved regions of the human immunodeficiency virus type 1 (HIV-1) genome. These were then employed in the microplate assay that involves the capture of target nucleic acid by virtue of its complementarity to an immobilized capture oligonucleotide. Hybridization is detected by incubation with a second specific signal oligonucleotide using an enzyme-dependent amplification system to yield a colorimetric readout. This strategy has been applied to detection of HIV DNA and RNA sequences in both model systems and clinical specimens.

Analytic Sensitivity

In an initial set of experiments in which a series of DNA samples containing known amounts of HIV DNA were amplified and then assayed in the nonradioactive system, fewer than 10 copies of HIV proviral DNA could be detected (Figure 2). These data suggested that application of this technology to the problem of HIV detection in clinical samples might allow the identification of individuals who harbor low levels of HIV proviral or viral nucleic acid, *e.g.*, prior to seroconversion.

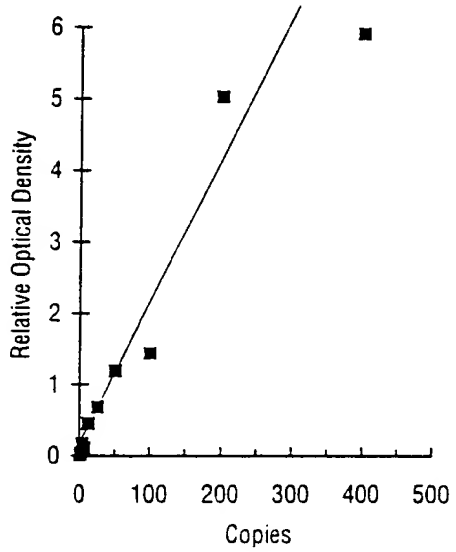
Figure 2
Detection of HIV Sequences in Amplified Samples

Samples represented 1 µg of human DNA that was amplified for 35 rounds in the presence of the indicated number of copies of cloned HIV DNA. Relative optical density was read at the termination of the detection reaction and normalized to 2 µl of the undiluted amplification reaction product. In all cases ODs of greater than 1.00 were based upon assays of diluted samples that gave OD readings between 0.1 and 1.0.

Sample	Copies	Relative Optical Density
2393	800,000	93.45
2410	80,000	38.28
2398	8,000	15.38
2391	800	8.74
2390	400	5.91
2395	200	5.03
2400	100	1.44
2394	50	1.19
2403	25	0.682
2397	12.5	0.455
2399	6.25	0.104
2402	3.12	0.170
2392	1.56	0.038
2396	0.00	0.014
water	0.00	0.003

Figure 3
Analytic Sensitivity

The data in Figure 2 are represented graphically



Low Levels of HIV Detected

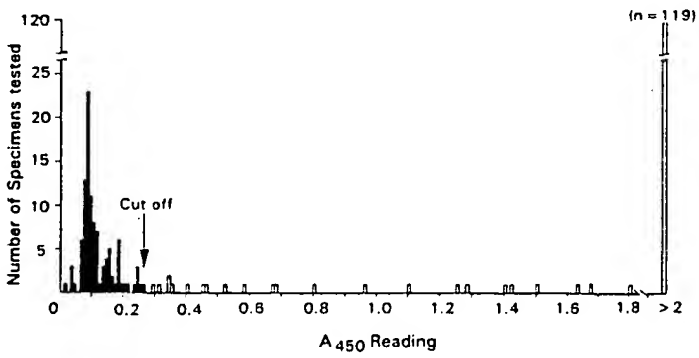
When enzymatically amplified HIV DNA, from either the *gag* or *env* regions was the target, the assay was able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. Furthermore, the assay detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted. These results confirm the applicability of the assay for the early detection of low levels of HIV.

Clear Cut-off Point

In any clinical assay the negative cut-off value, or the point at which a sample can be determined to be positive, is extremely important. To investigate this, 104 seronegative and 142 seropositive individuals were examined. Amplified DNA from these individuals was measured by the microplate assay and the results are shown in Figure 4 below.

Figure 4

Samples from positive and negative specimens plotted to demonstrate the clear negative cut off value obtained with the assay.



All DNA samples from negative individuals tested negative. The average ± 3SDs (99% confidence level) of the 104 negative specimens was 0.113 ± 0.156. The mean + 3SD (0.269) was used as the cut-off. Of the 142 positive specimens 84% gave readings >2 . Thus, as can be seen in Figure 4, the microplate assay can provide a clear discrimination between positive and negative individuals.

References:

Cook, A.F. et al. (1988) Synthesis and hybridization of a series of biotinylated oligonucleotides. Nuc. Acid Res. 16:4077-4095

Rapier, J.M. et al. (1993) Nonradioactive, colorimetric microplate hybridization assay for detecting amplified human immunodeficiency virus DNA. Clin. Chem. 39:244-247

Lee, L.S. et al. (1990) A nonisotopic hybrid capture assay for HIV nucleic acid sequences. Abstract US-CAP Academy of Pathology, March 1990.

Other Microplate Hybridization Assays are available from Enzo:

for HIV Type 2 detection:
HIV-2 Microplate Hybridization Assay **Cat. No. 46360**

for Tuberculosis detection:
MTB Microplate Hybridization Assay **Cat. No. 46340**

for Hepatitis B virus detection:
HBV Microplate Hybridization Assay **Cat. No. 46350**

Oligonucleotide pairs

for use with the microplate hybridization assays:

SK 38/SK 39 Oligonucleotide pair
complementary to HIV-1 gag region **Cat. No. 46331**

MTB 10/MTB 11 Oligonucleotide pair
complementary to MTB sequences **Cat. No. 46341**

HB01/HB02 Oligonucleotide pair
complementary to HBV core region **Cat. No. 46351**

VB306/VB310 Oligonucleotide pair **Cat. No. 46361**
complementary to HIV-2 sequences

Now available for direct detection of hepatitis B serum DNA!!!!!

Our Enhanced HBV DNA Microplate Hybridization Assay utilizes a high-sensitivity streptavidin-horseradish peroxidase for greater detection of specimens with less target DNA. Results can be quantified for copy number with Titration Standards.

HBV Enhanced
Microplate Hybridization Assay **Cat. No. 46353**

HBV Serum Specimen Titration Standards **Cat. No. 46354**

For further information on Enzo's Microplate Hybridization Assays or any of Enzo's other products:

Call:
516-694-7070
800-221-7705
Toll Free *within the Continental U.S. and Canada*
Between the hours of 8:30 AM - 5:30 PM Eastern Time

Fax:
516-694-7501

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60 Executive Boulevard
Farmingdale, New York 11735

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